THE PREVALENCE AND INTENSITY OF *PERKINSUS MARINUS* FROM THE MID NORTHERN GULF OF MEXICO, WITH COMMENTS ON THE RELATIONSHIP OF THE OYSTER PARASITE TO TEMPERATURE AND SALINITY

THOMAS M. SONIAT
DEPARTMENT OF BIOLOGICAL SCIENCES AND CENTER FOR BIO-ORGANIC STUDIES
UNIVERSITY OF NEW ORLEANS, LAKEFRONT, NEW ORLEANS, LA. 70148

and

JULIE D. GAUTHIER
DEPARTMENT OF BIOLOGICAL SCIENCES
UNIVERSITY OF NEW ORLEANS, LAKEFRONT, NEW ORLEANS, LA. 70148

Abstract

Twenty-three samples of oysters were collected from 19 sites along the mid Northern Gulf of Mexico and assayed for the prevalence and intensity of the parasite *Perkinsus marinus*. Prevalence of infection (measured as percent infection, PI) ranged from 0 to 100%, whereas intensity of infection (measured as weighted incidence, WI = sum of disease code numbers/number of oysters) ranged form 0.00 to 3.06. WI was correlated with water salinity (Kendall Tau Beta correlation coefficient = 0.331, $P < 0.05$), but not with water temperature.

Introduction

Perkinsus marinus (Mackin, Owen, and Collier) is a protistan parasite of the American oyster, *Crassostrea virginica* (Gmelin). It was first described as *Dermocystidium marinum* by Mackin, Owen, and Collier (1950) because of its close similarity to the freshwater parasitic fungus *Dermocystidium salmonis* Davis. Later observations indicated that *D. marinum* gave rise to gliding cells on "mucoid tracks" similar to those of slime molds and was reclassified as *Labyrinthomyxa marina* (Mackin and Ray 1966). Although ultrastructural studies by Perkins (1969) revealed a likeness of the parasite to the fungi, no cytoplasmic extensions or rhizoids were observed. Levine (1978) renamed the parasite *Perkinsus marinus* on the basis of electron microscopic work by Perkins (1976) which revealed the presence of an apical complex in a motile zoospore stage (Table 1).

P. marinus is a major cause of oyster mortality along the Gulf and Atlantic coasts (Mackin et al. 1950, Ray and Mackin 1955, Andrews 1965). Histological studies of oysters infected with the parasite show areas of inflamation and tissue destruction (Mackin 1951). Significant weight loss of diseased oysters has been documented (Ray et al. 1953), and disease intensity and levels of stress in oysters have been positively correlated Soniat and Koenig 1982).

Numerous studies (e.g., Mackin et al. 1950, Ray et al. 1953, Mackin 1955, Quick and Mackin 1971, Ogle and Flurry 1980, Soniat 1985) have shown that *P. marinus* is most prevalent during the warm months in high salinity areas. Oysters are more susceptible to the disease during spawning season which, along the Gulf Coast, continues throughout the summer (Mackin et al. 1950). However, high weighted incidence values (WI, a measure of disease intensity) have been shown to be directly related to high temperatures (Hewatt and Andrews 1955, Quick 1971). Although positive correlations have been made between salinity and WI, low salinity apparently imposes no physiologi-

TABLE 1. The various names and suggested taxonomic affinities of the oyster parasite.

Name	Affinities	Reference
Dermocystidium marinum	Protista	Mackin et al. (1950)
	Entomophthorales (Fungi)	Sparrow (in Ray, 1954a)
	Haplosporidia (Protozoa)	Sprague (1954)
	Chytridiales (Fungi)	Mackin & Boswell (1956)
	Saprolegniales (Fungi)	Perkins & Menzel (1967)
Labyrinthomyxa marina	Labyrinthulales (Slime mold)	Mackin & Ray (1966)
Perkinsus marinus	Apicomplexa (Protozoa)	Levine (1978)

cal handicap on the development of *P. marinus* (Ray 1954a); freshwater influx may simply dilute infective elements causing lower WI values (Mackin 1962). Stronger correlations have been found between WI and the product temperature and salinity (TxS) than between temperature or salinity alone (Soniat 1985).

As part of studies of oyster ecology and genetics, as well as effects of oil and gas operations upon oysters, we have had the opportunity to collect oysters and sample parasites from numerous locations in Texas, Louisiana, and Mississippi. This paper further documents a positive correlation between WI, salinity, and TxS values. It also provides current information on distribution, prevalence, and intensity of *P. marinus* along the Mid-Gulf Coast region.

Material and Methods

Oysters were collected from August 1985 to January 1987 at 13 sites along the mid Gulf Coast (Figure 1). Temperature (mercury thermometer) and salinity (American Optics refractometer) were recorded at each site. Small pieces of mantle tissues (~4mm^2) from 9 to 30 oysters of approximately commercial sizes (6.0-12.5 cm umbo-to-bill distance) were examined for parasitism by *P.*

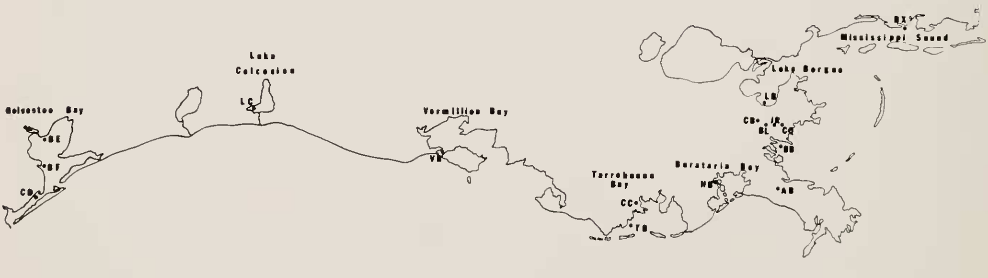

Fig. 1. A map of the mid northern Gulf of Mexico showing the sample sites. CO = Confederate Reef, AF = April Fool Reef, BE = Beasley's Reef (Galveston Bay); LC = Lake Calcasieu; VB = Vermilion Bay; TB = Terrebonne Bay; CC = Cocodrie; HB = Hackberry Bay (Barataria Bay area); AB = Adams Bay; BB = Black Bay; CB = Couchon Bay; BL = Bayou Linge; JR = Lake Jean Robin; CQ = Lake Coquille; LB = Lake Borgne; BX = Biloxi.

marinus using the Ray (1966) technique. The tissues were placed for one week in culture tubes containing a fluid thioglycollate medium and antibiotics (chloromycetin and mycostatin). Microscopic examination of the tissues revealed enlarged *P. marinus* presporangia upon treatment with iodine. The level of infection of each oyster was represented by a disease code number (Quick and Mackin 1971) which ranged as an integer from 0 (uninfected) to 6 (heavily infected). Percent infection (PI, a measure of disease prevalence) and weighted incidence were calculated for each site. Weighted incidence (Mackin 1962) was determined as follows:

$$WI = \frac{\text{sum of disease code numbers}}{\text{total number of oysters}}$$

The temperature/salinity interaction term (TxS) was also calculated for each site (Soniat 1985).

Non-parametric correlations (Kendall Tau Beta correlations) between temperature, salinity, TxS, WI, and PI were determined using the Statistical Analysis System package (Helwig and Council 1979) installed on a VAX 8600 computer at the University of New Orleans.

Results and Discussion

Temperatures varied from 10.5°C at Confederate Reef (CO1, Table 2) to 30.4°C at Terrebonne Bay (TB1); however, the average value was 21.1°C and most of the samples were taken when water temperatures were above 20°C. Salinity values varied more broadly. They ranged from 4 ppt at Vermilion Bay (VB1) to 32 ppt at Confederate Reef (CO2) with an overall mean of 16 ppt. The TxS was used to measure the potential of the environment to produce and sustain heavy levels of infection. The factor was developed by Soniat

TABLE 2. Data for temperature (°C), salinity (ppt), weighted incidence (WI), percent infection (PI), the product of temperature and salinity (TxS), and the number of samples (N) collected from the various sites. Site names and locations are given in Figure 1.

Site	Date	Temperature	Salinity	WI	PI	TxS	N
AB1	8/18/86	30.0	20	1.70	90	600.0	10
AF1	8/24/85	27.4	13	2.90	100	356.2	30
BB1	11/7/85	18.0	11	1.33	53	198.0	30
BE1	8/24/85	27.5	8	0.03	3	220.0	30
BL1	12/11/86	12.8	16	0.90	60	204.8	10
BX1	11/8/86	25.0	20	2.20	100	500.0	10
BX2	10/14/86	24.2	20	2.27	9	484.0	11
BX3	9/2/86	28.0	25	2.00	93	700.0	15
BX4	9/25/85	27.4	5	2.07	100	137.0	30
CB1	10/7/86	27.5	19	0.60	30	522.5	10
CC1	10/16/86	21.1	12	2.89	89	253.2	9
CO1	1/27/87	10.5	16	0.00	0	168.0	10
CO2	8/7/86	30.0	32	1.80	93	960.0	30
CO3	8/23/85	27.0	30	3.06	100	810.0	30
CQ1	12/11/86	12.8	16	1.10	70	204.8	10
CQ2	12/3/86	14.0	14	1.90	90	196.0	20
CQ3	12/11/86	13.0	16	1.90	90	208.0	10
HB1	11/19/85	25.0	9	0.00	0	225.0	30
JR1	12/11/86	12.8	16	1.60	90	204.8	10
LB1	10/8/85	10.9	10	0.17	13	209.0	30
LC1	12/17/85	11.0	11	1.20	60	121.0	30
TB1	9/12/85	30.4	16	1.27	53	486.4	30
VB1	11/30/85	22.8	4	0.00	0	91.2	30

(1985) as a simple method to account for the combined efforts of temperature and salinity since the parasite is more prevalent when high temperatures and high salinities co-occur (Mackin et al. 1950). Soniat (1985) showed a closer correlation between WI and TxS than between WI and salinity (temperature and WI were not correlated). Furthermore, salinity and temperature are sometimes inversely correlated (Soniat 1985), which confounds the interpretation of WI data in relation to these environmental variables. In the present study temperature and salinity were not correlated (Table 3). TxS values ranged from 91.2 at Vermilion Bay (VB1) to 960.0 from Confederate Reef (CO2). The highest WI value (3.06) was found at Confederate Reef (CO3), which is also where the second highest TxS value (810.0) was found. No infected oysters were found at Confederate Reef (CO1 on 1/27/87), Hackberry Bay (HB1), or Vermilion Bay (VB1). All oysters were found infected (PI = 100%) at April Fool Reef (AF1), Biloxi (BX1 on 11/8/86), and Confederate Reef (CO3 on 8/23/85). The lowest WI values (0.00-0.03) were found at sites with salinities that ranged from 4 ppt (VB1) to 9 ppt (HB1)—with two exceptions. The first exception was found at Confederate Reef (CO1) where the WI was 0.00 and the salinity was 16 ppt; however, the temperature was 10.5°C—the lowest recorded (Table 2). The low temperature could explain the low WI value; however, Ray (personal communication) found a WI of 1.62 on 1/6/87 (12°C, 14 ppt) and a WI of 0.84 on 2/17/87 (13°C, 20 ppt) from Confederate Reef oysters. The second exception was a Biloxi sample (BX4) which had a relatively high WI (2.07) and a low salinity (5 ppt). This sample was taken shortly after a freshet event and the intensity of infection probably did not have time to decrease from its typically high level.

TABLE 3. Kendall Tau Beta correlation coefficients and probability values for correlations between temperature (TEMP), salinity (SAL), weighted incidence (WI), percent infection (PI), and the product of temperature and salinity (TxS). N = 23.

	SAL	WI	PI	TxS
TEMP	0.200 0.199	0.202 0.185	0.204 0.191	0.564 <0.001
SAL	—	0.331 <0.050	0.354 <0.050	0.653 <0.001
WI	—	—	0.707 <0.001	0.321 <0.050
PI	—	—	—	0.314 <0.050

The typical WI pattern of decreasing parasitism with decreasing salinity was found at three reefs in Galveston Bay. Low (Beasley's), moderate (April Food), and high salinity (Confederate) reefs were sampled within a week of each other (the temperature difference was only 0.4°C). Beasley's Reef (BE1) had a WI of 0.03, April Fool Reef (AF1) had a WI of 2.90, and Confederate Reef (CO3) had a WI of 3.06 (Table 2). Details of the sample sites and information on *P. marinus* in Galveston Bay are provided by Hofstetter (1977). Ray (1987) finds consistently high levels of infection at Confederate Reef. The reef may serve as a refuge for the parasite in the Galveston Bay complex. Confederate Reef may also be a permanent source of infective agents (Ray, personal communication) since oysters there are not subject to freshets which would help control the parasite. Studies of oyster spat on Confederate and a nearby reef (Ray, 1987) have, in fact, caused Ray to revise earlier contentions (Mackin 1951, Ray 1954b) that young oysters (especially those less than a year old) are not susceptible to infection by *P. marinus*. Oysters may need a threshold inoculum of infective agents. The threshold is not only a function of age (or the time the oysters are exposed to infective agents), but also a function of the number of infective agents in the environment (or the proximity of oysters to the focus of infection). If oysters do become infected at earlier ages in areas near a focus of infection, then WI and PI data from oysters by size or age classes would be useful in locating disease "hot spots". Ray's work (Ray, 1987) confirms the results of Hofstetter (1971, 1977) who found *P. marinus* infections in spat from reefs in central Galveston Bay. Craig and Powell (1986) and Powell (personal communication) sampled oysters at 50 sites from Brownsville, Texas to the Florida Everglades, including 4 sites in Galveston Bay. Prevalence of the parasite was never less than 50% from sites in Texas; however, Craig and Powell did not sample any low-salinity reefs in Trinity Bay (from Beasley's Reef we found a PI of 3% and a WI of 0.03).

Mackin (1962) sampled oysters from more than 100 bays and bayous in Louisiana and found prevalences ranging from 0 to 100%. Some of his stations are near those sampled in the present study. Although *P. marinus* was discovered in Louisiana, where most of the early work was conducted, little recent information exists on the occurrence and distribution of the parasite there. Craig and Powell (1986) and Powell (personal communication) sampled nine Louisiana stations; Ray (1982) reports disease data from Lake Calcasieu; and Turner (1985) talks about *P. marinus*, but did not assay for the parasite.

The Lake Calcasieu site was in West Cove in the immediate vicinity of Ray's (1982) Station T-5 and Turner's (1985) Station W. Ray's (1982) samples from the area (taken on 11/7/82) showed higher WI values (2.1 vs. 1.2), and higher PI levels (83% vs 60%), but were taken at a higher temperature (14.4°C vs 11°C) and a higher salinity (20.1 ppt vs 11 ppt).

The Vermilion Bay location (VB1) had the lowest salinity, lowest TxS value, and a WI of 0 (Table 2). Mackin (1962) sampled the adjacent Atchafalaya Bay and likewise found no evidence of parasitism. Freshwater discharge from the Atchafalaya River maintains low salinities in both the Vermilion and Atchafalaya Bays, and *P. marinus* is not prevalent in either.

At the two sites established in the Terrebonne Bay area, WI values were 1.27 (TB1) and 2.89 (CC1). These levels of infection are similar to those found by Mackin (1962) who sampled numerous bays and bayous in the Terrebonne/Timbalier Bay area.

No infection (Table 2) was recorded in oysters from Hackberry Bay (HB1, NW section of the Barataria Bay complex). This bay is a relatively low salinity area (9 ppt on 11/19/85), where Mackin (1962) also did not detect the parasite. In contrast, the lower portions of Barataria Bay often have severe problems with *P. marinus* (Dugas, personal communication); Mackin (1962), for example, found a WI of 4.38 from gapers (dead oysters with the shells still attached and the meats intact) at Bayou Rigaud (Grand Isle).

Adams Bay was sampled numerous times by Mackin (1962); WI values ranged from 0 to 1.00. In the present study (AB1, Table 2) a WI of 1.70 was found.

The seven samples taken immediately east of the Mississippi River (BB1, BL1, CB1, CQ1-CQ3, JR1) had WI values from 0.90 to 1.90. These values were higher than those of Mackin (1962), who found a range of 0.00 to 1.77 in his samples taken east of the Mississippi River. With the present data it is not possible to definitively state that the area is experiencing increased problems with *P. marinus*; however, with an increase in salinity in this area (due to the intrusion of salt water since the construction of the Mississippi River Gulf Outlet) one could, *a priori*, expect increased problems with the parasite.

The Lake Borgne (LB1) samples were taken from an area that supports a few low salinity reefs (see Mackin and Hopkins 1962). Salinity, at the time of the sample, was 10 ppt and WI was low—0.17.

A single station in Mississippi was sampled four times (BX1-BX4). WI ranged from 2.00 to 2.27; salinity varied from 5 to 25 ppt, and temperature values were 24.2°C to 28.0°C (Table 2). Thus, temperature and WI values were relatively uniform, although salinity varied greatly. However, as mentioned previously, the BX4 sample (9/25/85) was taken immediately after a freshet, and almost certainly represents a WI typical of higher salinities. Ogle and Flurry (1980) sampled four reefs in Mississippi Sound over a 25 month period and found a low prevalance and intensity of the parasite. The greatest WI was 0.88—substantially lower than that of the present study.

Temperature was not correlated with salinity, WI, or PI (Table 3). Soniat (1985) found a significant inverse correlation between temperature and salinity from a single reef (April Fool Reef, Galveston Bay), which was also sampled for *P. marinus*. No correlation was found between temperature and WI and PI in the present study because temperatures did not vary greatly and most of the samples were taken at temperatures greater than 20°C. When temperatures are consistently high and salinity varies, salinity appears to be the controlling factor of the disease. Likewise, when salinities are relatively constant and temperatures are more variable, temperature is likely to be correlated with WI. In the present study and in Soniat (1985), salinity is correlated with WI and PI. These results are exactly opposite to those of Quick and Mackin (1971) who found no relationship between WI and salinity, and a significant relationship between WI and temperature. WI and PI are correlated with each other and both are correlated with the TxS interaction term (Table 3). In contrast to the work of Soniat (1985), temperature (in the present study) did not add to the significance of the TxS term. In fact, the TxS term is not as strongly correlated with WI as is salinity alone. Nonetheless, the TxS interaction term is a useful method of measuring the potential of the environment to sustain high levels of infection by *P. marinus*—especially when the sampling design includes seasonal sampling, and sampling of broad geographical areas, and when temperature and salinity are correlated with one another.

Acknowledgments

We are grateful to Roy Ary, Marc Breaux, Rick Howell, Livingston Lanclos, Martin Mayer, Al Segura, Lorene Smith, Andy Terpak, and Susan Vreeland for their assistance in the collection of oysters. The authors are also grateful to Janet Forbes for typing, and Jerilyn Green for proof reading. The project was primarily funded by the U.S. Fish and Wildlife Service and the UNO Fisheries Initiative Program.

Literature Cited

Andrews, J. D. 1965. Infection experiments in nature with *Dermocystidium marinum* in Chesapeake Bay. Chesap. Sci. 6: 60-67.

Craig, M. A. and E. N. Powell. 1986. A survey of *Perkinsus marinus* in Gulf of Mexico. Presented at th 78th Annual Natl. Shellfish. Assoc. meeting, June 22-26, Seattle, Washington.

HELWIG, J. T. and K. A. COUNCIL. 1979. SAS user's guide. SAS Institute Inc., Cary, N.C. 494 p.

HEWATT, W. G. and J. D. ANDREWS. 1955. Temperature control experiments on the fungus disease, *Dermocystidium marinum*, of oysters. Proc. Natl. Shellfish. Assoc. 46: 129-133.

HOFSTETTER, P. 1971. Galveston Bay oyster studies: 1971. Texas Parks and Wildl. Dept. Coastal Fish Proj. Rept. 1971, 107-124.

HOFSTETTER, R. P. 1977. Trends in population levels of the American oyster, *Crassostrea virginica* Gmelin on public reefs in Galveston Bay, Texas. Texas Parks and Wildl. Dept. Tech. Ser. No. 24. 99 p.

LEVINE, N. D. 1978. *Perkinsus* gen. n and other new taxa in the Protozoan phylum Apicomplexa. J. Parasitol. 64: 549.

MACKIN, J. G. 1951. Histopathology of infection of *Crassostrea virginica* (Gmelin) bv *Democystidium marinum* Makin, Owen and Collier. Bull Mar. Sci. Gulf and Caribb. 1: 15-30.

MACKIN, J. G. 1955. *Dermocystidium marinum* and salinity. Proc. Natl. Shellfish Assoc. 46: 116-128.

MACKIN, J. G. 1962. Oyster disease caused bv *Dermocystidium marinum* and other microorganisms in Louisiana. Publ. Inst. Mar. Sci. Univ. Texas 7: 132-299.

MACKIN, J. G. and J. L. BOSWELL. 1956. The life cycle and relationships of *Dermocystidium marinum*. Proc. Natl. Shellfish Assoc. 46: 112-115.

MACKIN, J. G. and S. H. HOPKINS. 1962. Studies on oyster mortality in relation to natural environments and to oil fields in Louisiana. Publ. Inst. Mar. Sci. Univ. Texas 7: 1-131.

MACKIN, J. G. and S. M. RAY. 1966. The taxonomic relationship of *Dermocystidium marinum*. Mackin, Owen and Collie J. Invert. Pathol. 8: 544-545.

MACKIN, J. G. H. M. OWEN, and COLLIER. 1950. Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). Science 111: 328-329.

OGLE, J. and K. FLURRY. 1980. Occurrence and seasonality of *Perkinsus marinus* (Protozoa:Apicomplexa) in Mississippi oysters. Gulf Res. Rept. 6: 423-425.

PERKINS, F. 1969. Ultrastructure of vegetative stages in *Labyrinthomyxa marina* (=*Dermocystidium marinum*), a commercially significant oyster pathogen J. Invert. Pathol. 13: 199-222.

PERKINS, F. O. 1976. Zoospores of the oyster pathogen, *Dermocystidium marinum*. I. Fine structure of the conoid and other sporozoan-like organelles. J. Parasitol. 62: 959-974.

PERKINS, F. O. and R. W. MENZEL. 1967. Ultrastructure of sporulation in the oyster pathogen *Dermocystidium marinum* J. Invert. Pathol. 9: 205-229.

QUICK, J., JR. 1971. Pathological and parasitological effects of elevated temperatures on the oyster *Crassostrea virginica* with emphasis on the pathogen *Labrinthomyxa marina*. *In* J. A. Quick, Jr. ed. A preliminary investigation: the effect of elevated temperature on the American oyster *Crassostrea virginica* (Gmelin). p. 105-171. Fla. Dept. Nat. Resources. Mar. Res. Lab. Prof. Paper Ser. No. 15.

QUICK, J. A, JR. and J. G. MACKIN. 1971. Oyster parasitism by *Labyrinthomyxa marina* in Florida. Fla. Dept. Nat. Resour. Mar. Res. Lab. Prof. Paper. Ser. No. 13. 1-55.

RAY, S. M. 1954a. Biological studies of *Dermocystidium marinum* a fungus parasite of oysters. Rice Inst. Pamphlet Monogr. Biol. Spec. Issue, 114 p.

RAY, S. M. 1954b. Studies on the occurrence of *Dermocystidium marinum* in young oysters. Proc. Natl. Shellfish Assoc. 44: 80-92.

RAY, S. M. 1966. A review of the culture method for detecting *Dermocystidium marinum* with suggested modifications and precautions. Proc. Natl. Shellfish. Assoc. 54: 55-69.

RAY, S. M. 1982. Summary report on monitoring program in Lake Calcasieu, Louisiana Ray Biological Company, Galveston, Texas.

RAY, S. M. 1987. Salinity requirments of the American oyster, *Crassostrea virginica*. *In* A. J. Mueller and G. A. Matthews, eds. Freshwater inflow needs of the Matagorda Bay system with focus on the needs of penaeid shrimp p. E1-E29. NOAA Technical Memorandum MNFS-SEFC.

RAY, S. M. and J. G. MACKIN. 1955. Studies of pathogenesis of *Dermocystidium marinum*. Proc. Natl. Shellfish Assoc. 45: 164-167.

RAY, S. M., J. MACKIN, and J. L. BOSWELL. 1953. Quantitauve measurement of the effect on oysters of disease caused by *Dermocystidium marinum* Bull Mar. Sci. Gulf and Caribb. 3: 6-33.

SONIAT, T. M. 1985. Changes in levels of infection of oysters by *Perkinsus marinus*, with special references to the interaction of temperature and salinity upon parasitism. Northeast Gulf Sci. 7: 171-174.

SONIAT, T. M. and M. L. KOENIG. 1982. The effects of parasitism by *Pekinsus marinus* on the free amino acid composition of *Crassostrea virginica* mantle tissue. J. Shellfish Res. 2: 25-28.

SPRAGUE, V. 1954. Protozoa. *In* P. S. Galtsoff., ed., Gulf of Mexico: its origin, waters and marine life. U.S. Fish Wildlife Ser. Fish Bull. Vol. 89. p. 243-256.

TURNER, H. M. 1985. Parasites of eastern oysters from subtidal reefs in a Louisiana estuary with a note on their use as indicators of water quality. Estuaries 8: 323-325.